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Major Article

Evaluation of the ability of different detergents and disinfectants to remove and kill organisms in traditional biofilm

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Background: The objective of this study was to assess the ability of different detergent and disinfectant combinations to eradicate bacteria in traditional biofilm.

Methods: *Enterococcus faecalis* and *Pseudomonas aeruginosa* were used to develop biofilm over 8 days. The biofilm on each minimum biofilm eradication concentration peg contained 8 $\log_{10}$ colony forming units (CFU)/cm^2 of both bacteria. The detergents evaluated were as follows: Prolystica Enzymatic 2X, Prolystica Neutral 2X, Neodisher, and Endozime Bio-Clean. The disinfectants evaluated were as follows: glutaraldehyde, accelerated hydrogen peroxide, and ortho-phthalaldehyde. Biofilm removal was evaluated using viable count, protein and carbohydrate quantitation, and scanning electron microscopy.

Results: Only Prolystica Enzymatic 2X and Endozime Bio-Clean killed both *E faecalis* (3.90 $\log_{10}$ CFU/mL reduction) and *P aeruginosa* (3.96 $\log_{10}$ CFU/mL reduction) in suspension. None of the detergents tested could provide >1 $\log_{10}$ CFU/cm^2 reduction for bacteria within biofilm. Any combination of detergent and high-level disinfectant reduced the level of both *E faecalis* and *P aeruginosa* within biofilm by 3-5 $\log_{10}$ CFU/cm^2. Although the combination of Endozime Bio-Clean and glutaraldehyde provided a 6 $\log_{10}$ reduction, it could not eliminate both bacteria within biofilm.

Conclusions: Our data indicate that if biofilm accumulates in flexible endoscope channels during repeated rounds of reprocessing, then neither the detergent nor high-level disinfectant will provide the expected level of bacterial removal or killing.

Flexible endoscopes are widely and increasingly used for diagnostic and therapeutic procedures.[1-3] For example, gastrointestinal endoscopy is the gold standard for the diagnosis of gastric cancer, gastric ulcer, duodenal ulcers, and gastrointestinal conditions worldwide.[1,4] Flexible endoscopes are classified as semicritical devices that come in contact with mucous membranes or nonintact skin[1,4] and as such should be sterilized or receive a minimum of high-level disinfection (HLD).[1,4] Although low temperature sterilization using plasma has been validated for some flexible endoscopes with short channels, the flexible endoscope with longer channels (eg, those used for gastrointestinal procedures) requires ethylene oxide sterilization, which has long aeration requirements (eg, 18-24 hours). As such, the most commonly used approach to flexible endoscope reprocessing involves cleaning (generally with enzymatic detergent), followed by HLD with a liquid chemical disinfectant, rinsing, drying, and storage.[4,5]

The complex design of flexible endoscopes with narrow lumens (that may contain an elevator wire) and crevices makes them extremely difficult to clean.[2] Changes in the integrity of endoscope surfaces resulting from frequent use can hinder the removal of organic material and microorganisms during reprocessing.[4,6] Because of their complexity, frequent use, and narrow margin of safety during reprocessing, flexible endoscopes pose unique challenges for infection control.[4,7,8] A single contaminated endoscope may be used hundreds of times each year and as such has the potential to infect or colonize a large number of patients. The level of surface alterations on the internal channel surface increases during repetitive use (each endoscope is typically used for 5-10 years), and the risk of

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contamination increases with the age of the endoscope.[9] Recent reports of multidrug-resistant organisms transmitted as a result of contaminated duodenoscopes have demonstrated high transmission and infection rates.[10,11] Many of these reports have suggested that persistent survival of multidrug-resistant organisms in reprocessed flexible endoscopes may be caused by biofilm formation.[10,12,13]

Bacteria residing within biofilm are many times more resistant to chemical inactivation than bacteria in suspension, and this could affect the efficacy of HLD.[2,5,6,12] It has been estimated that 65% of nosocomial endogenous infections directly involve biofilms,[2] but the rate of exogenous infections from biofilm contamination of flexible endoscopes is not truly known. Formation of biofilm inside endoscope channels can result in failure of endoscope reprocessing and is an important factor in the transmission of endoscopy-related infections.[10,12,13] Recent outbreaks[10,11] have led some to suggest that the current manufacturer's instructions for cleaning and decontamination may be unable to fully remove or kill biofilm. There are limited data regarding the impact of various detergents and high-level disinfectants on biofilm.

The objective of this study was to assess the ability of 4 different detergents and 3 different high-level disinfectants that are frequently used for endoscope reprocessing to remove or kill organisms in traditional biofilm.

MATERIALS AND METHODS

Organic challenge: Artificial test soil and bacteria

In this research study, *Pseudomonas aeruginosa* (ATCC 15442; ATCC, Manassas, VA) and *Enterococcus faecalis* (ATCC 29212; ATCC) were used. The bacteria were cultured on Tryptic Soy Agar with 5% sheep blood (BA; Oxoid, Toronto, ON, Canada) at 35°C-39°C aerobically for 24 hours. Each of the test bacteria were suspended in Artificial Test Soil (ATS) (Artificial Test Soil: US patent 6,447,990)[14] to achieve approximately 10^8 colony forming units (CFU)/mL. The ATS bacterial concentration was determined using 1:10 serial dilutions in sterile phosphate-buffered saline (sPBS), and 0.1 mL of each dilution was inoculated onto BA and spread over the surface. Inoculated plates were incubated at 35°C-39°C aerobically for 24 hours, and the colonies were counted.

Biofilm model used for simulated-use testing

Biofilm was formed using a hydroxyapatite-coated MBEC Biofilm Inoculator (Innovotech, Edmonton, AB, Canada) following the manufacturer's instructions.[15] Hereafter, the hydroxyapatite-coated MBEC Biofilm Inoculator will be referred to as the HMBEC (Fig 1).

The ability to form biofilm was tested using a modification of the traditional biofilm method. The ATS bacteria suspension was used for inoculation of the 96 well tray, and the HMBEC pegs were exposed to this suspension for 48 hours at room temperature (RT) with rocking action (8 full back and forth rocks per minute). After 48 hours, the HMBEC pegs were rinsed with sterile tap water 3 times (30 seconds for each rinse) and then fed with fresh ATS bacteria for 2 hours at RT with rocking. This procedure was repeated 3 times a day followed by ATS bacteria exposure for another 48 hours at RT. The traditional biofilm was developed over 8 days and was designed to mimic multiple rounds of organic exposure followed by prolonged biofilm formation to mimic daily use followed by days and weekend of storage that were similar to what might happen to flexible endoscopes. There was no high-level disinfectant used during traditional biofilm development (worst-case biofilm development).

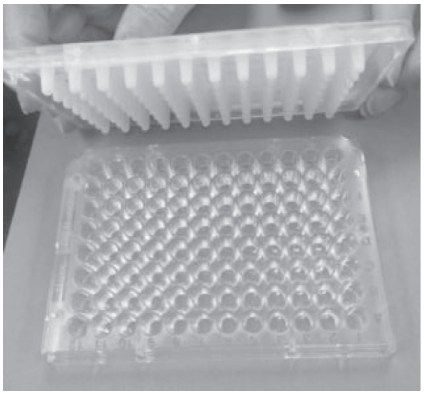

Fig 1. Hydroxyapatite-coated MBEC Biofilm Inoculator model. The lid has 96 identical polystyrene pegs that fit into a standard 96-well microtiter plate. Each well is inoculated with growth medium and microorganisms, and the pegs on the lid are inserted into the liquid in the wells. Biofilm is formed on the pegs by rocking this tray at the desired temperature. The media in each well is replenished every 24-48 hours to ensure continued microbial replication. Each peg can be aseptically removed for analysis of the biofilm.

Extraction of HMBEC pegs for analysis

After the 8-day biofilm accumulation protocol, individual pegs were aseptically snapped off the lid using a sterile hemostat and placed in a sterile snap cap test tube (Simport, Quebec, QC, Canada) containing 500 µL double-strength neutralizer (Tween 80 [SIGMA, St. Louis, MO] [6% wt/vol], lecithin [0.6% wt/vol], sodium thiosulfate [1.0% wt/vol], and L-histidine [0.2% wt/vol])[16] and 500 µL sterile reverse osmosis (sRO) water. The suspensions were then mixed using a Finemixer SH200 rocking mixer (Yangchun, Seoul, Korea) for 2 minutes, sonicated at 50/60 Hz using a Bransonic 1200 Ultrasonic clear (Branson Canada, Pickering, ON, Canada) for 5 minutes, and vortexed for 1 minute. This is the extraction procedure recommended in the MBEC manufacturer instructions for use. Each experiment was performed using 5 replicate pegs.

Detergents and disinfectants test

The use dilution, exposure time, and temperature provided by the detergent and high-level disinfectant manufacturers are listed in Table 1. Each time that a high-level disinfectant was used, the minimum effect concentration was evaluated using the specific manufacturer's recommended test.

Benchmarks for adequate manual cleaning

The manual cleaning benchmarks for flexible endoscope channels that were established by Alfa et al[17] were used. If manual cleaning was adequate, then there should be <6.4 µg/cm^2 of protein, <1.79 µg/cm^2 of carbohydrate, and <4 $\log_{10}$ CFU/cm^2 of viable microorganisms.

Detergent and disinfectant control tests

Suspension detergent test

Suspension testing was used to compare the ability of each detergent (Table 1) to kill organisms in bacterial suspension with and without neutralizer. The bacterial control for (detergent-sRO water) consisted of a sterile test tube containing 1,800 µL of sRO water but no neutralizer and no detergent. The bacterial control for (detergent-neutralizer) consisted of a sterile test tube containing 1,800 µL of neutralizer[16] but no sRO water and no detergent. For the detergent testing, a sterile tube containing 900 µL of each detergent (at